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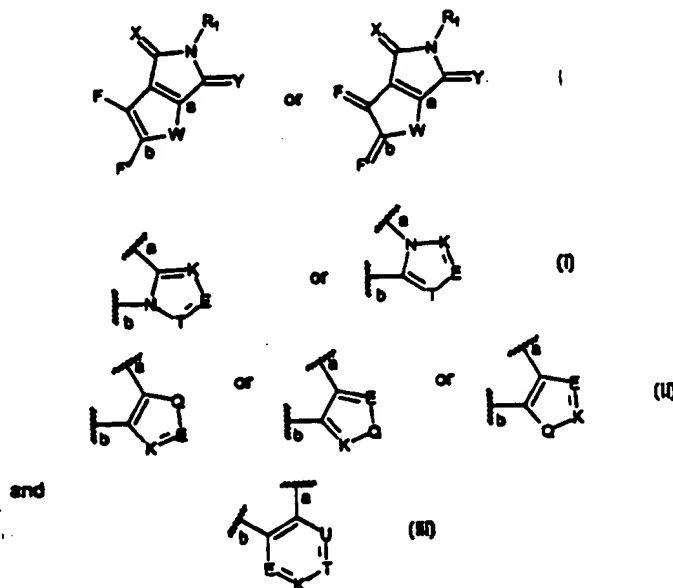
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(54) Title: GRANULATIMIDE DERIVATIVES FOR USE IN CANCER TREATMENT



(57) Abstract

Novel granulatimide compounds and pharmaceutical formulations thereof are provided. Compounds of this invention have general formula (I) or (II) wherein are independently R or Z as defined below, or in combination F and F' is Ar₁ as defined below; Ar₁ is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings, optionally substituted with R or Z; W is selected from the group consisting of formula (i); (ii) or (iii), wherein the structures are as described in: (i), (ii) and (iii). R and Z are optional substituents as defined in the application.

GRANULATIMIDE DERIVATIVES FOR USE IN CANCER TREATMENT

BACKGROUND

Natural products are a rich source of novel organic compounds, many of which have interesting and desirable biological activities. From extracts of an organism of interest, such as marine invertebrates, methods of chemical separation and analysis may be applied to elucidate the structure of biologically active compounds. These chemical structures then form the basis for synthetic modifications to enhance the desired activity.

Marine ascidians, or sea squirts, have a number of unique secondary metabolites with potent biological activity. Colonial ascidians within the family *Didemnidae* have been particularly prolific, containing nitrogenous amino acid derived metabolites, including various cyclic peptides. The compounds termed didemnimides were isolated from *Didemnum conchylatum*. These compounds are indole-maleimide-imidazole alkaloids, which could hypothetically be synthesized as a condensation of tryptophan and histidine.

The further study of *Didemna* products, and the characterization of their active agents, is of particular interest for the development of novel therapeutic agents and uses thereof.

Relevant Literature

Didemnimide compounds are described by Vervoot *et al.* (1997) J. Org. Chem. 62:1486-1490. In describing synthesis of such didemnimide compounds, Terpin *et al.* (1998) Tetrahedron 54:1740-1752 reported a ring closing event that may occur upon irradiation or upon recrystallization from methanol of a Boc protected intermediate used in the didemnimide synthesis scheme.

Known G2 checkpoint inhibitors include purine analogues, e.g. caffeine, pentoxifylline, 2-aminopurine, 6-dimethylaminopurine; and staurosporine with its derivative UCN-01 (7-hydroxystaurosporine). See, for example, Busse *et al.* (1978) Radiat. Res. 76:292-307; Schlegel (1986) Science 232:1264-1266; Downes *et al.* (1990) J. Cell. Biol. 110:1855-1859; Steinmann *et al.* (1991) P.N.A.S. 88:6843-6847; Andreasson *et al.* (1992) P.N.A.S. 89:2272-2276; Tam *et al.* (1992) Cell Growth Differ. 3:811-817; and Wang *et al.* (1996) J. Nat'l Cancer Inst. 88:956-965.

granulatimide and γ -irradiation do not kill p53+ cells synergistically (method identical to (B) except that MCF-7 cells are used).

Figure 2 is a graph showing G2 checkpoint inhibition by granulatimide.

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DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Novel granulatimide compounds of Formula I are provided. This invention includes the naturally occurring compounds, granulatimide and iso-granulatimide, in purified or partially purified form, including extracts containing these compounds
10 taken from naturally occurring sources. In one embodiment of the invention, formulations of the compounds in combination with a physiologically acceptable carrier are provided.

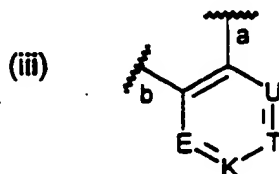
The pharmaceutical formulations are useful as a cytotoxic agent; and to inhibit protein kinases, including those that act to regulate the G2 checkpoint.
15 Such G2 checkpoint inhibition prevents arrest, or releases cells that are arrested at this checkpoint, thereby permitting the cells to proceed to mitosis. This invention also provides the use of compounds of Formula I to sensitize cells to the effects of DNA damaging agents; and the use of such compounds in the formulation of agents, including medicaments, for potentiating the effect of DNA damaging
20 agents on cells.

DEFINITIONS

It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents
25 described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

As used herein the singular forms "a", "and", and "the" include plural
30 referents unless the context clearly dictates otherwise. Thus, for example, reference to "a compound" includes a plurality of such compounds and reference to "the protein" includes reference to one or more proteins and equivalents thereof known to those skilled in the art, and so forth. All technical and scientific terms

in which K and E are independently selected from the group consisting of: N, CR and CZ, and wherein R, Z and Q are as defined below; and



in which K, E, T and U are independently selected from the group consisting of: N, CR and CZ, and wherein R and Z are as defined below;

R₁ is selected from the group consisting of: R; RCO-; Ar₂CO-; and, Ar₂CH₂-

wherein Ar₂ is an aromatic substituent selected from the group consisting of: phenyl, naphthyl, anthracyl, phenanthryl, furan, pyrrole, thiophene, benzofuran, benzothiophene, quinoline, isoquinoline, imidazole, thiazole, oxazole, and pyridin, and Ar₂ may be optionally substituted with R or Z, wherein R and Z are as defined below;

R is selected from the group consisting of H; and a structural fragment having a saturated or unsaturated linear, branched, or cyclic, skeleton containing one to ten carbon atoms in which the carbon atoms may be optionally substituted with a substituent selected from the group consisting of: -OH; -OR₃; -O₂CR₃; -SH; -SR₃; -SOCR₃; -NH₂; -NHR₃; -NH(R₃)₂; -NHCOR₃; -NRCOR₃; -I; -Br; -Cl; -F; -CN; -CO₂H; -CO₂R₃; -CHO; -COR₃; -CONH₂; -CONHR₃; -CON(R₃)₂; -COSH; -COSR₃; -NO₂; -SO₃H; -SOR₃; and -SO₂R₃, wherein R₃ is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

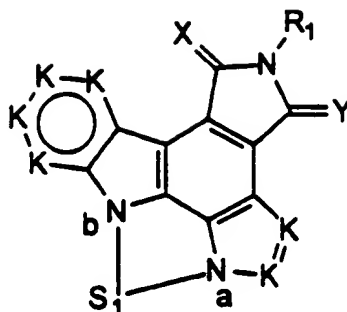
Z is an optional substituent selected from the group consisting of: H; -OH; -OR; -O₂CR; -SH; -SR; -SOCR; -NH₂; -NHR; -NH(R)₂; -NHCOR; -NRCOR; -I; -Br; -Cl; -F; -CN; -CO₂H; -CO₂R; -CHO; -COR; -CONH₂; -CONHR; -CON(R)₂; -COSH; -COSR; -NO₂; -SO₃H; -SOR; and, -SO₂R;

Q is selected from the group consisting of: NR₁; O; S, and C(R)₂; and

X and Y are independently selected from the group consisting of: O; H, OH; and H₂.

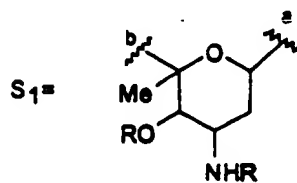
In a preferred embodiment of the invention, F and F' are a bicyclic aromatic system comprising a five-membered and a six-membered ring, having the structure as follows:

Formula III

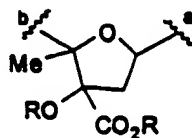


Where S1 is selected from the group consisting of

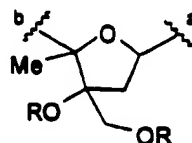
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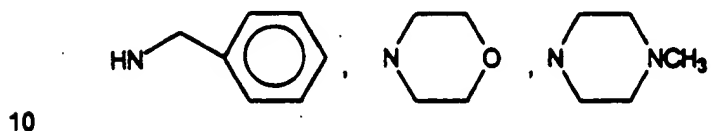


wherein K, X, Y and R₁ are as defined above, and R is an alkyl group of from 1 to 6 carbon atoms, branched or unbranched, linear or circular.

where K, X, Y and R₁ are as defined above, and S₃ is



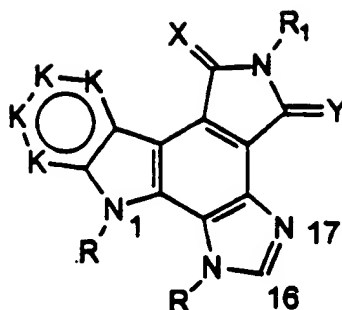
and X₁ is selected from the group of N(CH₃)₂, NHCH₃, NH₂, ,



or S₃ is a linear bridge of between five and eight carbon, nitrogen or oxygen atoms, where the bridge carbon atoms may have OR or NR₂ substituents and the bridge nitrogen atoms may have R substituents, where R is as defined above.

15 Preferred structures for W are the five membered rings described above, more preferably comprising one or two nitrogen atoms. Preferably, E, K, T and U (where present) are: N or CH. Also, preferably Q is NH. Also preferably, R₁ is: H or CH₃. Also preferably, X and Y are oxygen.

20 The following are examples of compounds of Formula 1 in which F and F' are Ar₁, with each of K, Q, R and Z being independently selected as defined above.

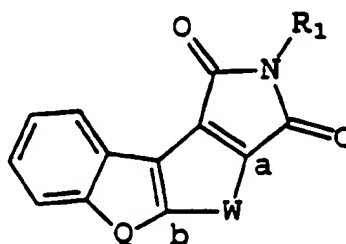


Formula II

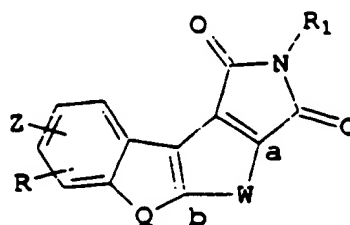
Preferably, excluded as granulatinimide compounds of the invention are derivatives comprising a protecting group, *e.g.* Boc, *etc.*, at the position corresponding to 1 in the above scheme.

Preferred compounds of Formula I are as follows, with substituents as defined above or as particularly defined below, where "Me" represents a methyl substituent throughout.

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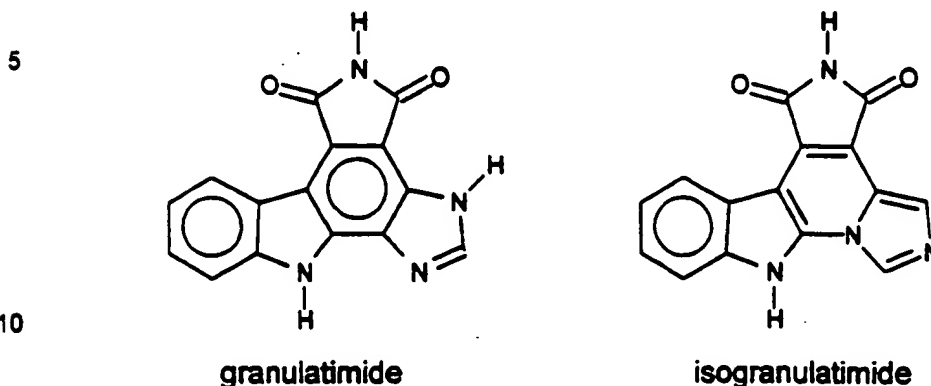
wherein Q=NH; R₁=H; and each of K, E, T = N or CH;



200

where in Q=NH; R₁=H; and each of K, E, T = N or CH;

In one embodiment of the invention, the granulativimide compound is one of the naturally occurring compounds: granulativimide, or isogranulativimide.



Pharmaceutically acceptable salts of the granulativimide compounds also fall within the scope of the compounds as disclosed herein. The term "pharmaceutically acceptable salts" as used herein means an inorganic acid addition salt such as hydrochloride, sulfate, and phosphate, or an organic acid addition salt such as acetate, maleate, fumarate, tartrate, and citrate. Examples of pharmaceutically acceptable metal salts are alkali metal salts such as sodium salt and potassium salt, alkaline earth metal salts such as magnesium salt and calcium salt, aluminum salt, and zinc salt. Examples of pharmaceutically acceptable ammonium salts are ammonium salt and tetramethylammonium salt. Examples of pharmaceutically acceptable organic amine addition salts are salts with morpholine and piperidine. Examples of pharmaceutically acceptable amino acid addition salts are salts with lysine, glycine, and phenylalanine.

25

Protein Kinases and Inhibitors: These enzymes use the gamma phosphate of ATP or GTP to generate phosphate monoesters utilizing protein alcohol groups on serine or threonine, and/or protein phenolic groups (tyrosine) as phosphate group acceptors. They are related by virtue of their homologous kinase domains, which consist of 200-300 amino acid residues. The kinase domains impart the catalytic activity by binding and orientation of the phosphate donor as a complex with divalent cation; binding and orientation of the polypeptide substrate; and transfer of the γ -phosphate from the ATP or GTP to the acceptor hydroxyl residue.

G2 ch ckpoint. Normal cells respond to DNA damage in one of two ways, depending upon their type and the degree of damage. The cells may activate an apoptotic pathway leading to suicide of the cell and its removal or, survival of a damaged cell may be promoted by activating checkpoints that temporarily halt the normal cycle of growth and division to allow time for DNA repair. The checkpoints operate during the G1 phase of the cycle so that DNA is repaired before it is replicated in the S phase; and, during the G2 phase so that DNA is repaired before chromosomes are segregated in the mitosis phase (M).

The subject granulatimide compounds are active in the inhibition of the G2 checkpoint. They are of interest as therapeutic reagents to treat cancer, etc. Administration of the subject compounds will release cells from the G2 checkpoint; and can result in apoptosis of the treated cells. Preferably the treated cells are unable to activate the G1 checkpoint. Many human tumors have mutations in the protein p53. As a result, they are unable to activate the G1 checkpoint, but can still utilize the G2 checkpoint. Inhibiting the checkpoint would drive tumor cells into mitosis, thereby increasing the effectiveness of cytotoxic agents that act by damaging DNA, e.g. cisplatin, bleomycin, and etoposide; and radiotherapy.

Over 50% of human cancers exhibit a loss of function of the protein p53. Cells with mutated p53 are unable to activate the G1 checkpoint in response to DNA damage. However, the G2 checkpoint (although usually weaker than in normal cells) still provides an opportunity to repair the DNA damage before cell division. Inhibition of the G2 checkpoint alone generally does not have a strong effect on normal cells, or tumor cells that retain normal G1 checkpoint activity. G2 checkpoint inhibitors used in combination with a DNA damaging agent will significantly increase the killing of cells that cannot activate the G1 checkpoint.

Throughout this specification, the term "G2" or "G2 phase" means the phase of the cell cycle between the end of DNA synthesis and the beginning of mitosis. A cell in G2 has an interphase nucleus as determined by microscopy, and duplicated DNA (usually determined by flow cytometry).

G2 checkpoint Inhibitor. Throughout this specification, the term "G2 checkpoint inhibitor" means a substance which is capable of releasing a cell from

animals. Specific examples of suitable cells are the following: cells which have incorporated the human papillomavirus type-16 E6 gene, cell lines which are mutant for p53 function including Burkitt's human lymphoma cell line CA46 and the human colon carcinoma cell line HT-29 (CA46 and HT-29 are available from the American Type Culture Collection). Cell lines with a genetic deficiency which disrupts the G1 checkpoint other than by disruption of p53 may also be employed in this assay.

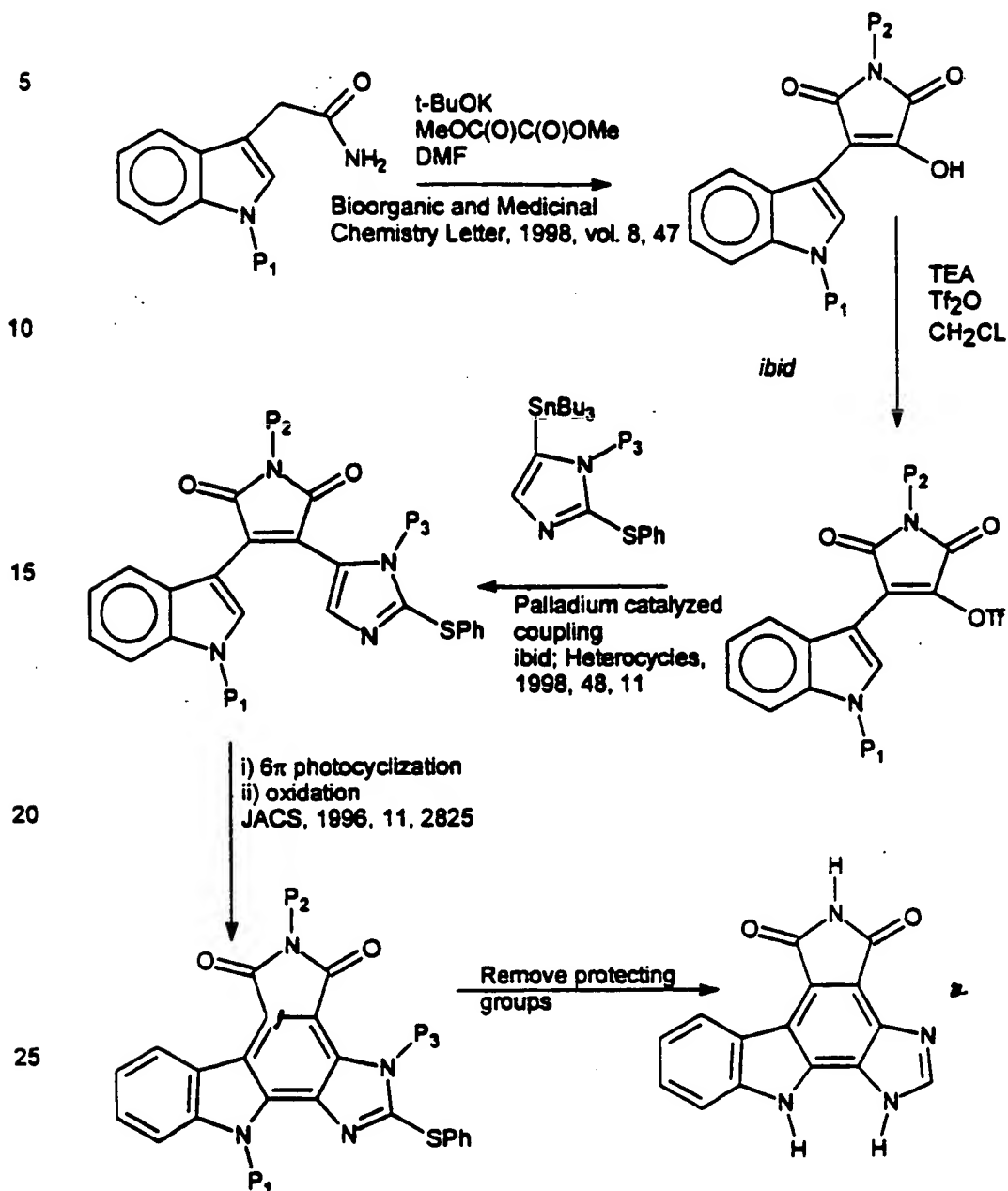
Once a cell line incapable of G1 arrest is chosen, conditions for arresting a majority of the cells at G2 in response to a DNA damaging agent are optimized by determining appropriate culture conditions, incubation time, and type and dosage of DNA damaging agent. Preferably, at least 50% of the cells in a culture will be arrested at G2 in response to the DNA damaging agent. Maximizing the proportion of cells in the population which are arrested at G2 will reduce the background signal.

Once the conditions for G2 arrest in the cell culture are established, the assay may be carried out in one of two ways. First, the cells may be arrested at G2 in response to the DNA damaging agent and then treated with the sample to determine whether there is release from G2 arrest or, the cells may be treated with the sample prior to the time when the majority of the cells would be expected to be arrested at G2 and determine whether the cells are prevented from G2 arrest.

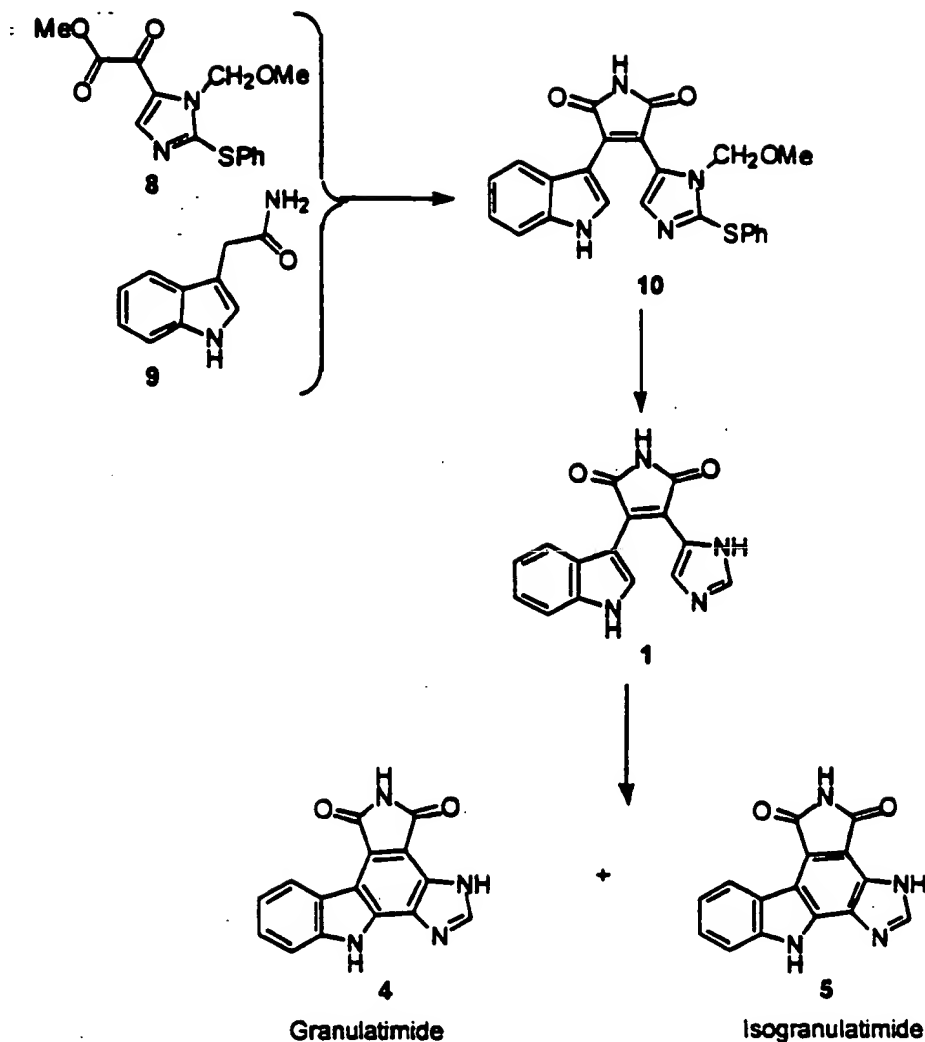
Release from G2 arrest or prevention of G2 arrest is detected by a quantitative determination of the cells which proceed to mitosis. The assay culture is treated with an agent which will arrest such cells in mitosis. Such agents include microtubule depolymerizing agents that arrest cells in metaphase, such as nocodazole. This will prevent cells from exiting mitosis and entering the next cell cycle.

In the assay, determination of the cells which proceed to mitosis is carried out using any of the known immunological methods by employing antibodies which have specificity for mitotic cells. Monoclonal antibodies demonstrating such specificity are known and include MPM-2 which was raised against mitotic HeLa cells and recognizes phospho-epitopes that are highly conserved in mitotic proteins of all eukaryotic species. Other examples are the monoclonal antibodies recognizing phospho-epitopes in the paired helical filament proteins (PHF) found in

An exemplary synthetic process is as follows, where P_1 , P_2 and P_3 are suitable protecting groups. The starting indole compound (appropriately substituted by R and or Z) may be made by methods known in the art.



An alternative synthesis scheme, outlined below, involves essentially a single coupling step to produce a didemnimide compound followed by cyclization to produce a compound of Formula I, wherein F, F', X and Y are as defined for Formula I, and W is as defined for Formula I joined to the reactant at (a) and not (b):



Alternatively, compounds of Formula I can be produced from granulativimide
 5 or isogranulativimide which may be synthesized as described above or obtained
 from natural sources as described in the Examples. The following schematic
 describes modification of granulativimide to produce derivatives having different X
 and Y substituents. The ratio of II to III will be dictated by the choice of protecting
 groups P₁, and P₂ or P₃, which may be (for example) Cbz, R or H. A protecting
 10 group will be used at either P₂ or P₃, depending upon the position of the double
 bond in the ring. The chemistry is described in Link, *et al.* (1996) J. American
 Chemical Society 118:2825, at p. 2832.

All Z and R groups can be added to aromatic carbons in W and Ar₁ prior to
 the coupling step to prepare the maleimide intermediates. All R, RCO, Ar₂CO, and

PHARMACEUTICAL FORMULATIONS

The compounds of this invention can be incorporated into a variety of formulations for therapeutic administration. More particularly, the compounds of the present invention can be formulated into pharmaceutical compositions by combination with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. As such, administration of the compounds can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, etc., administration. The active agent may be systemic after administration or may be localized by the use of regional administration, intramural administration, or use of an implant that acts to retain the active dose at the site of implantation.

In pharmaceutical dosage forms, the compounds may be administered in the form of their pharmaceutically acceptable salts. They may also be used in appropriate association with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

For oral preparations, the compounds can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

The compounds can be formulated into preparations for injections by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

The compounds can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can be formulated into

tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

The combined use of granulatimide compounds and other cytotoxic agents has the advantages that the required dosages for the individual drugs is lower, and the effect of the different drugs complementary. Depending on the patient and condition being treated and on the administration route, the granulatimide compounds may be administered in dosages of 0.1 μ g to 10 mg/kg body weight per day. The range is broad, since in general the efficacy of a therapeutic effect for different mammals varies widely with doses typically being 20, 30 or even 40 times smaller (per unit body weight) in man than in the rat. Similarly the mode of administration can have a large effect on dosage. Thus for example oral dosages in the rat may be ten times the injection dose. Higher doses may be used for localized routes of delivery.

A typical dosage may be a solution suitable for intravenous administration; a tablet taken from two to six times daily, or one time-release capsule or tablet taken once a day and containing a proportionally higher content of active ingredient, etc. The time-release effect may be obtained by capsule materials that dissolve at different pH values, by capsules that release slowly by osmotic pressure, or by any other known means of controlled release.

Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Some of the specific compounds are more potent than others. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means. A preferred means is to measure the physiological potency of a given compound.

For use in the subject methods, the granulatimide compounds may be formulated with other pharmaceutically active agents, particularly other anti-metastatic, anti-tumor or anti-angiogenic agents. Angiostatic compounds of interest include angiostatin, endostatin, carboxy terminal peptides of collagen alpha (XV), etc. Cytotoxic and cytostatic agents of interest include adriamycin, alkeran, Ara-C, BICNU, busulfan, CNNU, cisplatin, cytoxan, daunorubicin, DTIC, 5-FU, hydrea, ifosfamide, methotrexate, mithramycin, mitomycin, mitoxantron, nitrogen mustard, velban, vincristine, vinblastine, VP-16,

therapy, the active agents can be delivered together or separately, and simultaneously or at different times within the day.

The susceptibility of a particular tumor cell to killing with the combined therapy may be determined by *in vitro* testing. Typically a culture of the tumor cell is combined with a combination of a cytotoxic compound and a granulatinimid compound at varying concentrations for a period of time sufficient to allow the active agents to induce cell death, usually between about one hour and one week. For *in vitro* testing, cultured cells from a biopsy sample of the tumor may be used. The viable cells left after treatment are then counted.

The dose will vary depending on the specific cytotoxic agent utilized, type of tumor, patient status, etc., at a dose sufficient to substantially ablate the tumor cell population, while maintaining patient viability. In some cases therapy may be combined with stem cell replacement therapy to reconstitute the patient hematopoietic function. Treatment will generally be continued until there is a substantial reduction, e.g. at least about 50%, decrease in the tumor burden, and may be continued until there are essentially no tumor cells detected in the body.

All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the compounds and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtue of prior invention.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to the numbers used (e.g. amounts, temperature, concentrations, etc.) but some experimental errors and deviations should be allowed for. Unless otherwise indicated, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees centigrade; and pressure is at or near atmospheric.

peroxide was added for 1 hour at room temperature and the plates were read at 405 nm using a BioTek™ plate reader. Positive controls treated with 2 mM caffeine gave absorbance readings of about 1.0.

G2 checkpoint inhibition was detected in extracts from the ascidian
5 *Didemnum granulatum* (Subphylum Urochordata, Class Ascidiacea) collected from rocky, shallow water marine habitats along the coastline of southern Brazil.

Collection and Extraction of D. Granulatum.

Didemnum granulatum (85 g wet wt.) was collected during the summer of
10 1995 at depths of 1 m at the rocky shore of Araca beach, Sao Sebastiao (Sao Paulo state, southeastern Brazil). A second collection was made at the Arquipelago do Arvoredo (Santa Catarina state, southern Brazil, 150 g wet wt.) and in the Sao Sebastiao Channel (Sao Paulo state, 185 g wet wt.) during November 1997. Freshly collected animals were stored in ethanol at -20°C.
15 Animals obtained from the different collections were processed separately as follows: after decantation of ethanol, the animal was blended and extracted three times with methanol. The ethanol and methanol extracts were combined, filtered and evaporated *in vacuo* to give a gummy residue. The bulk of the residue was dissolved in 8:2 MeOH-CH₂Cl₂ and filtered for elimination of inorganic salts while
20 small amounts of the residue were dissolved in DMSO for use in the G2 checkpoint inhibition assay described above.

Isolation of Active Compounds.

Methanol extracts from the Brazilian ascidian *D. granulatum* were
25 fractionated by gel filtration on Sephadex LH-20 (eluent: MeOH) followed by reversed phase HPLC (eluent: acetonitrile/0.05% trifluoroacetic acid (1:1)) affording a series of eight fractions having different constituents by TLC. Only one fraction exhibited G2 checkpoint inhibition activity, which provided the novel alkaloid compounds later termed iso-granulatimide and granulatomide. Other
30 fractions contained various forms of the alkaloid didemnimide which were inactive. Didemnimide A and D were identified by comparison of NMR and MS data with literature values (Vervoort, H.C. *et al.* [supra]).

indole NH. The absence of a resonance in the ^1H NMR spectrum of the compound that may be assigned to the indole H-2 or H-3 protons indicated the presence of substituents at C-2 and C-3.

Very broad resonances at δ 8.10 and δ 9.12 in the ^1H NMR spectrum were assigned to the imidazole moiety. The broadness of the imidazole resonances was attributed to tautomeric equilibrium of the NH protons. The large chemical shift observed for the H-4 resonance (δ 8.51) in the new compound relative to the chemical shift observed for the H-4 resonance in didemnime A (δ 7.07) was attributed to a neighbouring group effect from a C-9 maleimide carbonyl.

As described above, the new compound was predicted to be a polycyclic aromatic differing from didemnime A by the presence of a bond between the C-2 carbon of the indole fragment and either C-14 or N-17 of the imidazole fragment. In the former case, the compound would be granulamide as described herein and in the latter case, isogranulamide. The broadness of the imidazole resonances in the NMR spectra initially made use of NMR ineffective in distinguishing the two compounds. Therefore, both granulamide and isogranulamide were synthesized as described herein and compared to the compound derived from the natural source which revealed the compound to be isogranulamide. TLC analysis (CH_2Cl_2 - MeOH 9:1; UV at 254nm) of pooled active fractions from the natural extract using synthetic granulamide as a reference revealed presence of the latter compound in an amount substantially less than isogranulamide. However, it was determined that the synthetic granulamide is very insoluble in most common organic solvents including ethanol and methanol and was therefore not efficiently extracted from the natural source in the procedure described above.

Granulamide is soluble in DMF or DMSO and one of the latter solvents is recommended for extraction of granulamide from the natural source.

Table 1 provides comparative ^1H -NMR data determined using synthetic compounds as recorded in $\text{DMSO}-d_6$.

would preferentially kill p53- tumors in humans treated with DNA damaging therapy.

As is shown in Figure 2, granulatinide may be more potent as a G2 checkpoint inhibitor ($IC_{50} = 1.3\mu\text{m}$) as compared to isogranulatinide.

5

Example 2

Synthesis of Isogranulatinide and Granulatinide.

Throughout these examples, TLC was carried out by using commercial aluminum-backed silica gel 60 plates. Flash chromatography was carried out with
10 230-400 mesh silica gel (E. Merck). THF was distilled from sodium/benzophenone and CH_2Cl_2 was distilled from CaH_2 . Commercial EtOH (reagent grade) and MeCN (HPLC grade) were used without further purification. Molecular sieves were dried under vacuum with heating for 5 hours prior to use. All reactions were carried out under an atmosphere of dry argon using glassware that had been
15 thoroughly flame dried.

Methyl 2-(1-methoxymethyl-2-phenylthioimidazol-5-yl)glyoxylate (8).

To a cold (-78°C), stirred solution of 1-methoxymethyl-2-phenylthioimidazole (Ohta, S. *et al.* (1992) Chem. Pharm. Bull. 40:2681) (340 mg, 1.55 mmol) in dry
20 THF (8.0 mL) was added a solution of n-BuLi (1.47 M in hexanes, 1.26 mL, 1.85 mmol) and the mixture was stirred for 1 hour. A solution of dimethyl oxalate (540 mg, 4.58 mmol) in dry THF (2.0 mL) was added and the mixture was stirred at -78°C for an additional 1.25 hours. The reaction mixture was treated with saturated aqueous NH_4Cl (5.0 mL) and Et_2O (20 mL). The phases were separated and the
25 aqueous layer was extracted with Et_2O (25 mL). The combined organic extracts were washed (brine, 10 mL), dried (MgSO_4), and concentrated. Flash chromatography (35 g of silica gel, 1:1 petroleum ether- Et_2O) of the crude material afforded 305 mg (66%) of 8 as a beige solid that exhibited mp $59-60^\circ\text{C}$; IR (KBr) 1729, 1657, 1305, 1273, 1167, 1114, 784 cm^{-1} ; ^1H NMR (CDCl_3 , 400 MHz) δ 8.23
30 (s, 1 H), 7.52-7.54 (m, 2 H), 7.37-7.38 (m, 3 H), 5.81 (s, 2 H), 3.91 (s, 3 H), 3.36 (s, 3 H); ^{13}C NMR (CDCl_3 , 100 MHz) δ 172.2, 161.6, 154.1, 145.4, 145.3, 133.2, 129.5, 129.2, 128.7, 75.8, 56.5, 53.0; HREIMS calcd for $\text{C}_{14}\text{H}_{14}\text{N}_2\text{O}_4\text{S}$ 306.0674, found 306.0674.

$J = 8.0$ Hz), 5.02 (s, 2 H), 3.07 (s, 3 H); ^{13}C NMR (100 MHz) δ 171.8, 171.7, 140.5, 136.5, 134.3, 132.9, 131.8, 124.3, 122.4, 121.7, 120.5, 120.3, 118.4, 112.3, 104.7, 76.4, 55.6; HREIMS calcd for $\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_3$ 322.1066, found 322.1066.

5 *Synthesis of Didemnimide A (1).*

To a stirred suspension of 11 (67.4 mg, 0.209 mmol) in CH_2Cl_2 (15.0 mL) at room temperature was added a solution of BBr_3 in CH_2Cl_2 (1.0 M, 2.1 mL, 2.1 mmol) and the deep blue solution was stirred for 5 hours. The mixture was treated with saturated aqueous NaHCO_3 (10 mL) and EtOAc (20 mL) and then was stirred
10 for 0.25 hours. The phases were separated and the aqueous layer was extracted with EtOAc (2 x 15 mL). The combined organic extracts were washed (brine, 10 mL), dried (MgSO_4) and concentrated. Flash chromatography (10 g of silica gel, 15:1 CH_2Cl_2 -MeOH) of the crude material afforded 15.8 mg of recovered 11 as well as 45.0 mg (77%, 100% based on recovered starting material) of 1 as an orange
15 solid ^1H NMR (400 MHz, major tautomer) δ 12.45 (br s, 1 H), 11.66 (br s, 1 H), 10.87 (br s, 1 H), 8.05 (s, 1 H), 7.71 (br s, 1 H), 7.68 (br s, 1 H), 7.39 (br d, 1 H, $J = 7.8$ Hz), 7.07 (br m, 2 H), 6.87 (br m, 1 H); ^{13}C NMR (100 MHz) δ 172.8, 172.6, 136.1, 135.9, 130.9, 130.5, 126.0, 126.0, 121.7, 121.3, 119.7, 119.2, 112.2, 111.5, 105.0; HREIMS calcd for $\text{C}_{15}\text{H}_{10}\text{N}_4\text{O}_2$ 278.0804, found 278.0804.

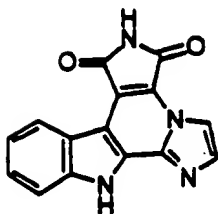
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Granulatimide (4) and Isogranulatimide (5).

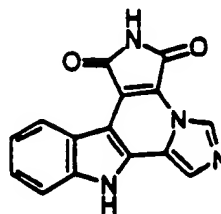
To a solution of 1 (12.9 mg, 0.046 mmol) in MeCN (5.0 mL) was added a catalytic amount of palladium-on-carbon (10% Pd) and the resulting mixture was sparged with argon for 0.5 hours. The mixture was irradiated (450 Watt Hanovia
25 medium pressure mercury vapor lamp, quartz reaction vessel) for 6.5 hours. The reaction mixture was filtered through Celite, the Celite was washed with DMF (10 mL), and the combined filtrate was concentrated. The remaining material was taken up in EtOAc (30 mL) and the resultant solution was washed (brine, 4 x 20 mL), dried (MgSO_4) and concentrated. Flash chromatography (20 g of silica gel,
30 10:1 CH_2Cl_2 -MeOH) of the crude material afforded 11.7 mg (91%) of 4 as a yellow solid and 1.0 mg (8%) of 5 as a red solid. Final purification was achieved on RPHPLC (1:1 CH_3CN -0.05% TFA). Granulatimide (4): ^1H NMR (400 MHz) see Table 1; UV (MeOH) 230, 270, 285, 295, and 380 nm; HREIMS calcd for

Exempl 4

Synthesis of Granulatimide Compounds.



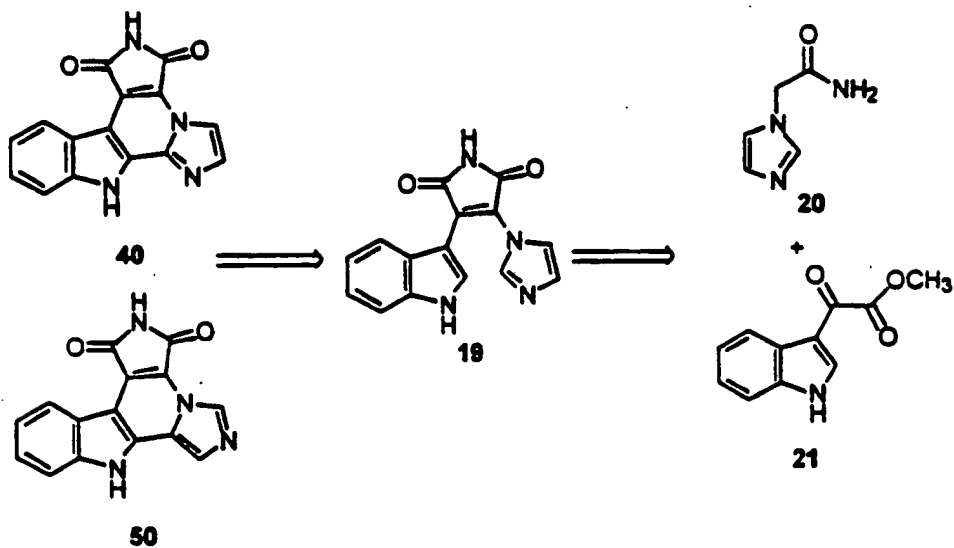
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50

5

Retrosynthesis



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P

solid that exhibited: ^1H NMR (400 MHz) δ 12.40 (bs, 1 H), 8.44 (d, 1 H, $J = 3.0$ Hz), 8.15 (d, 1 H, $J = 7.0$ Hz), 7.54 (d, 1 H, $J = 7.6$ Hz), 7.24–7.31 (m, 2 H), 3.88 (s, 3 H); ^{13}C NMR (100 MHz) δ 178.7, 163.9, 138.3, 136.7, 125.4, 123.8, 122.8, 121.1, 112.7, 112.4, 52.5; HREIMS calcd for $\text{C}_{11}\text{H}_9\text{NO}_3$ 203.0582, found 5 203.0582.

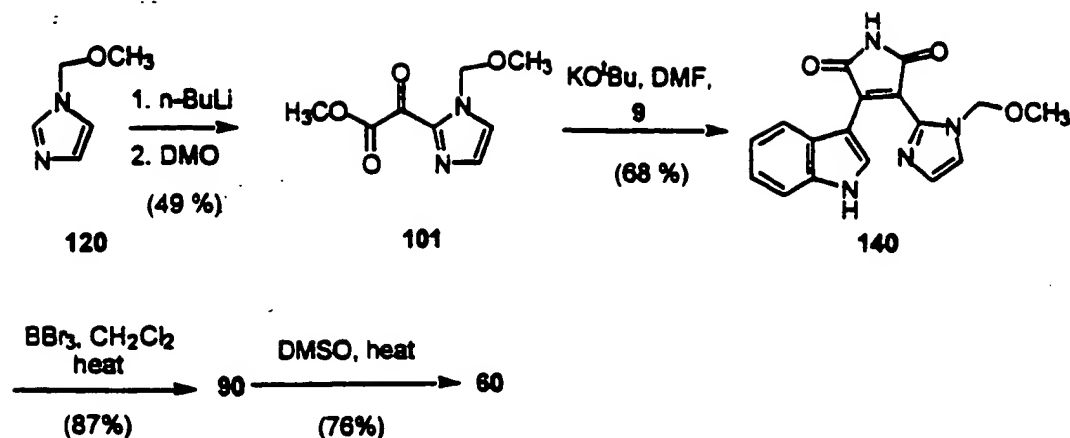
3-[4-(1H-imidazol-1-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indole (19).

To a stirred solution of *t*-BuOK (140 mg, 1.25 mmol) in DMF (2.0 mL) at 0 °C was added a solution of **20** (31.1 mg, 0.249 mmol) and methyl indole-3-glyoxylate (**21**) (101.1 mg, 0.498 mmol) in DMF (3.0 mL). The reaction mixture was stirred for 12 h at rt and then heated to 45 °C for 3 h. The reaction mixture was then cooled and treated with saturated aqueous NH_4Cl (5 mL) and EtOAc (20 mL). The phases were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with brine (4 x 10 mL), dried (MgSO₄), and concentrated. Flash chromatography (25 g of silica gel, 10:1 then 6:1 CH_2Cl_2 -MeOH) of the crude material afforded 54.5 mg (79%) of **19** as a red solid that exhibited IR (KBr) 3121, 2729, 1767, 1719, 1651, 1495, 1340, 1239, 746 cm^{-1} ; ^1H NMR (400 MHz) δ 10.75 (bs, 1 H), 10.00 (bs, 1 H), 8.03 (d, 1 H, $J = 3.1$ Hz), 7.78 (s, 1 H), 7.48 (d, 1 H, $J = 8.2$ Hz), 7.29 (s, 1 H), 7.11 (dd, 1 H, $J = 8, 8$ Hz), 7.04 (s, 1 H), 6.80 (dd, 1 H, $J = 8, 8$ Hz), 6.21 (d, 1 H, $J = 7.6$ Hz); ^{13}C NMR (100 MHz) δ 170.4, 168.4, 137.8, 136.4, 131.6, 128.8, 125.8, 124.3, 124.1, 122.5, 120.6, 120.1, 119.6, 112.3, 102.3; HREIMS calcd for $\text{C}_{15}\text{H}_{10}\text{N}_4\text{O}_2$ 278.0804, found 278.0804.

RAB-009 (40) and RAB-010 (50).

A solution of **19** in *t*-BuOH and MeCN was sparged with argon for 0.5 h. The yellow mixture was then irradiated (450 Watt Hanovia medium pressure mercury vapour lamp, quartz reaction vessel) for 4 h. The reaction mixture was concentrated and redissolved in acetone (10 mL). To the crude reaction product in acetone at rt was added 10 X CuCl_2 and the reaction mixture was stirred, exposed to air, overnight. The solvent was then removed in vacuo and the residue was taken up in DMF (10 mL) and filtered through celite. The filtrate was then diluted

Synthetic Scheme.



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Methyl 2-(1-methoxymethyl-1H-imidazol-2-yl)-glyoxylate (101).

To a cold (-48 °C), stirred solution of 1-methoxymethyl-1H-imidazole (**120**) (335 mg, 2.99 mmol) in dry THF (15.0 mL) was added a solution of *n*-BuLi (1.50 M in hexanes, 2.30 mL, 3.45 mmol) and the mixture was stirred for 1 h. The reaction mixture was then cooled to -78°C and added to a solution of dimethyloxalate (1.17 g, 9.9 mmol) in THF (10.0 mL) at -78°C. The mixture was stirred at -78°C for an additional 0.33 h. The reaction mixture was treated with saturated aqueous NH₄Cl (5 mL) and EtOAc (15 mL). The phases were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with brine (10 mL), dried (MgSO₄), and concentrated. Flash chromatography (50 g of silica gel, 50:1 CH₂Cl₂-MeOH) of the crude material afforded 290 mg (49%) of **101** as an oil that exhibited IR (KBr) 2957, 1744, 1679, 1412, 1276, 1215, 1109, 1005 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ 7.38 (s, 1 H), 7.33 (s, 1 H), 5.71 (s, 2 H), 3.96 (s, 3 H), 3.33 (s, 3 H); ¹³C NMR (CDCl₃, 100 MHz) δ 177.9, 164.3, 140.6, 132.9, 126.9, 78.8, 57.5, 53.4; HREIMS calcd for C₈H₁₀N₂O₄ 198.0641, found 198.0641.

3-[4-(1-methoxymethyl-1H-imidazol-2-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indole (140). To a stirred solution of **101** (142.0 mg, 0.717 mmol) and indol

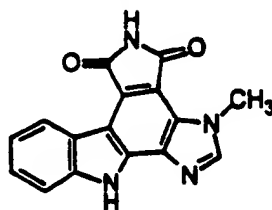
RAB-007 (60).

A stirred solution of 90 (14.4 mg, 0.052 mmol) in DMSO (5 mL) was heated to 120 °C for 8 h. The reaction mixture was cooled to rt, diluted with EtOAc (30 mL), washed with brine (3 X 10 mL), dried (MgSO₄), and concentrated. Flash chromatography (20 g of silica gel, 20:1 then 10:1 CH₂Cl₂-MeOH) of the crude material afforded 11.0 mg (76%) of 60 as an orange solid that exhibited IR (KBr) cm^{-1} 2600-3400, 1755, 1717, 1631, 1591, 1575, 1464, 1376, 1336, 1232, 751; ¹H NMR (400 MHz) δ 13.36 (bs, 1 H), 11.13 (bs, 1 H), 8.63 (d, 1 H, $J = 7.7$ Hz), 8.40 (s, 1 H), 7.93 (s, 1 H), 7.69 (d, 1 H, $J = 8.2$ Hz), 7.48 (dd, 1 H, $J = 7.7$ Hz), 7.38 (dd, 1 H, $J = 7.7$ Hz); HREIMS calcd for C₁₅H₈N₄O₂ 276.0647, found 276.0647.

In the G2 checkpoint inhibition assay, compound 60 had an IC₅₀ of 2 μM .

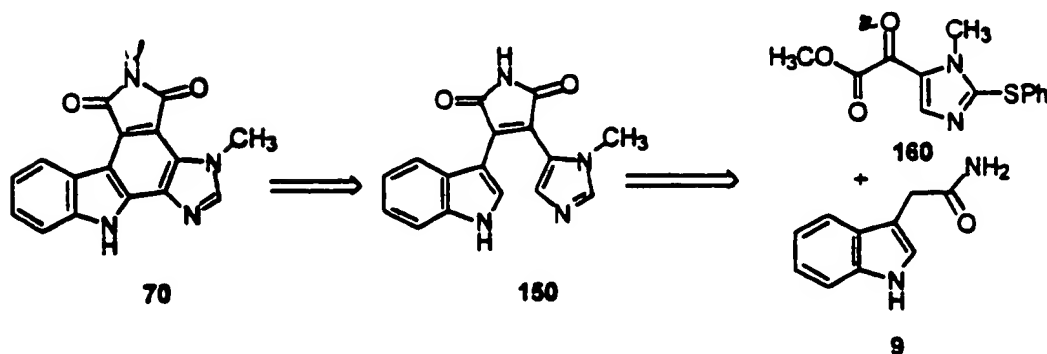
Example 6

Synthesis of Cyclodidemnimide C (70).



70

Retrosynthesis.



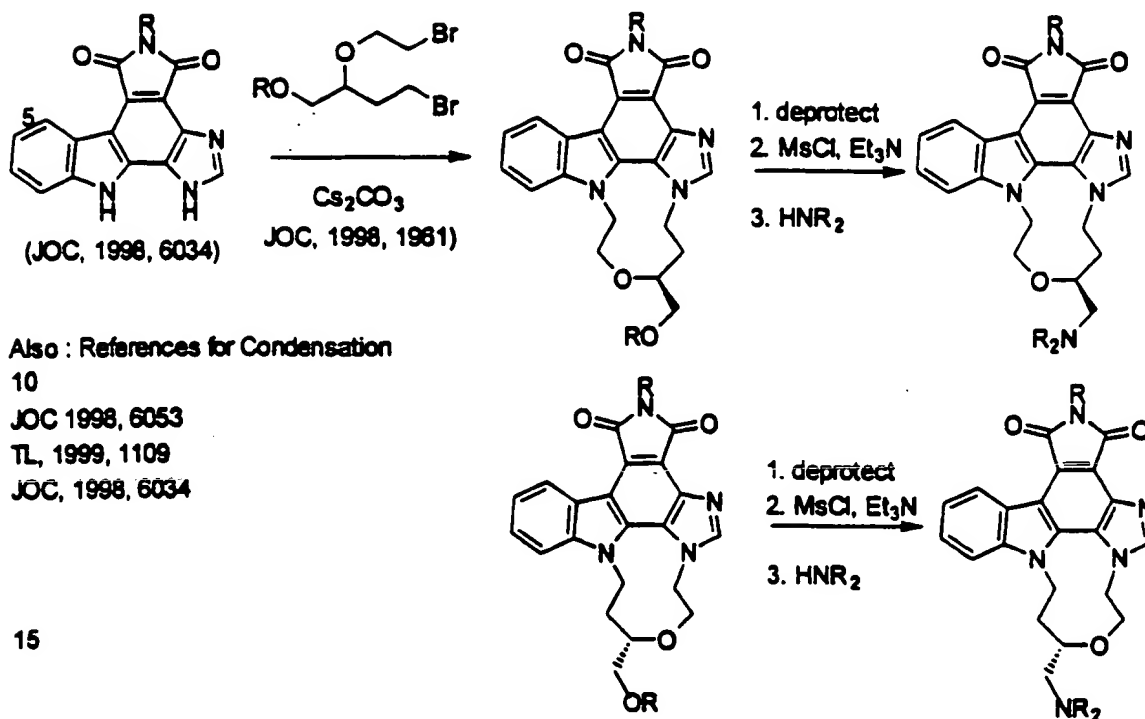
at it was added sequentially 4A molecular sieves (~ 50 mg) and a solution of **160** (28 mg, 0.10 mmol) and indole-3-acetamide **9** (22 mg, 0.13 mmol) in DMF (1.5 mL). The reaction mixture was heated to 40 °C and stirred for 48 h. The dark purple solution was treated with hydrochloric acid (1 N, 1.0 mL) and EtOAc (20 mL). The phases were separated and the aqueous layer was extracted with EtOAc (2 X 20 mL). The combined organic extracts were washed with brine (4 x 10 mL), dried (MgSO₄), and concentrated. Flash chromatography (20 g of silica gel, 30:1 CH₂Cl₂-MeOH) of the crude material afforded 35 mg (88%) of **170** as a red solid that exhibited; IR (KBr) 2500-3600, 1760, 1713, 1631, 1581, 1441, 1339, 744 cm⁻¹; ¹H NMR (400 MHz) δ 12.06 (bs, 1 H), 11.19 (bs, 1 H), 8.09 (d, 1 H, J = 2.1 Hz), 7.46 (d, 1 H, J = 8.0 Hz), 7.14-7.31 (m, 6 H), 7.03 (d, 1 H, J = 7.6 Hz), 6.81 (dd, 1 H, J = 8.8 Hz), 6.47 (d, 1 H, J = 8.1 Hz), 3.07 (s, 1 H); ¹³C NMR (100 MHz) δ 171.6, 171.4, 138.6, 136.4, 134.6, 133.7, 132.7, 131.8, 129.4, 127.4, 126.7, 126.8, 124.5, 120.8, 119.5, 117.9, 112.4, 104.8, 32.4; HREIMS calcd for C₂₂H₁₆N₄O₂S 400.0994, found 400.0994.

Didemnimide C (150).

To a refluxing solution of **170** (46.7 mg, 0.117 mmol) in EtOH (10 mL) was added Raney Ni (W-2, 50% slurry in water, ~120 mg) and the suspension was refluxed for 1 h. The reaction mixture was then cooled to rt and filtered through Celite. The Celite was washed with DMF (6 mL) and MeOH-TFA (100:1, 30 mL) and the combined organic washes were concentrated. The organic concentrate was then diluted with EtOAc (40 mL) and washed with NaHCO₃ (4 x 10 mL) brine (3 x 20 mL), dried (MgSO₄) and concentrated. Flash chromatography (25 g of silica gel, 10:1 CH₂Cl₂-MeOH) of the crude material afforded 26.4 mg (77%) of **150** as an orange solid that exhibited; IR (KBr) 3163, 3041, 1766, 1702, 1538, 1345, 1236, 1221, 748 cm⁻¹; ¹H NMR (400 MHz) δ 11.97 (bs, 1 H), 11.09 (bs, 1 H), 8.06 (d, 1 H, J = 2.6 Hz), 7.69 (bs, 1 H), 7.44 (d, 1 H, J = 8.1 Hz), 7.10 (dd, 1 H, J = 8.8 Hz), 6.78 (dd, 1 H, J = 8.8 Hz), 6.40 (d, 1 H, J = 8.1 Hz), 3.17 (s, 1 H); ¹³C NMR (100 MHz) δ 171.9, 171.7, 140.3, 136.4, 133.9, 132.0, 131.5, 124.7, 122.3, 120.5, 119.7, 112.2, 105.0, 32.0; HREIMS calcd for C₁₆H₁₂N₄O₂ 292.0960, found 292.0960

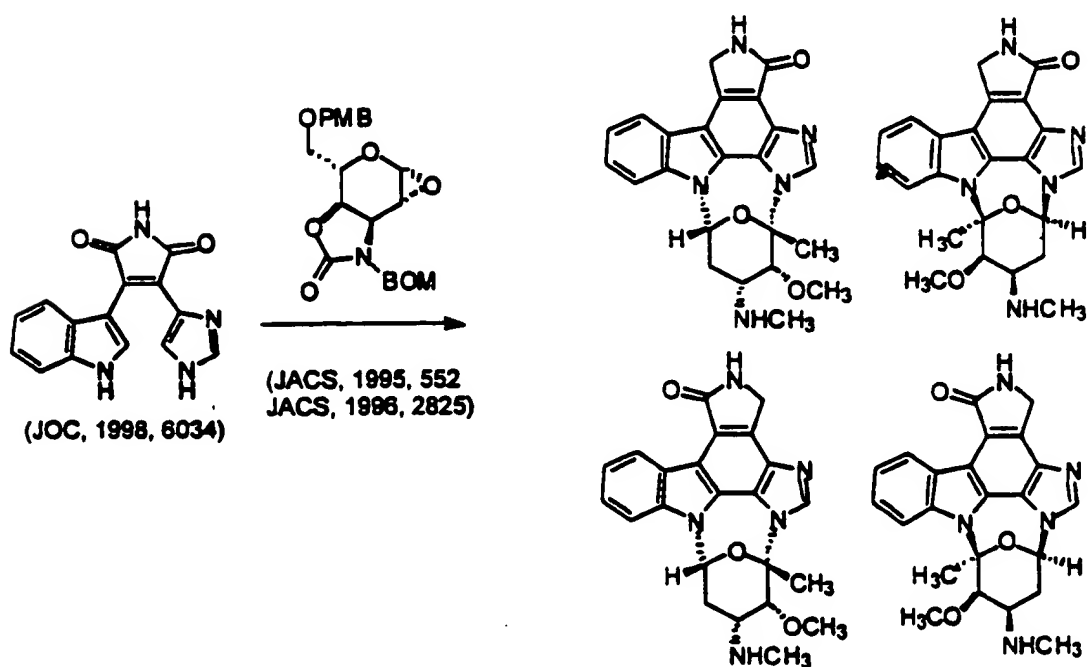
Exempl 8

Synth sis of Compounds related to granulimid .



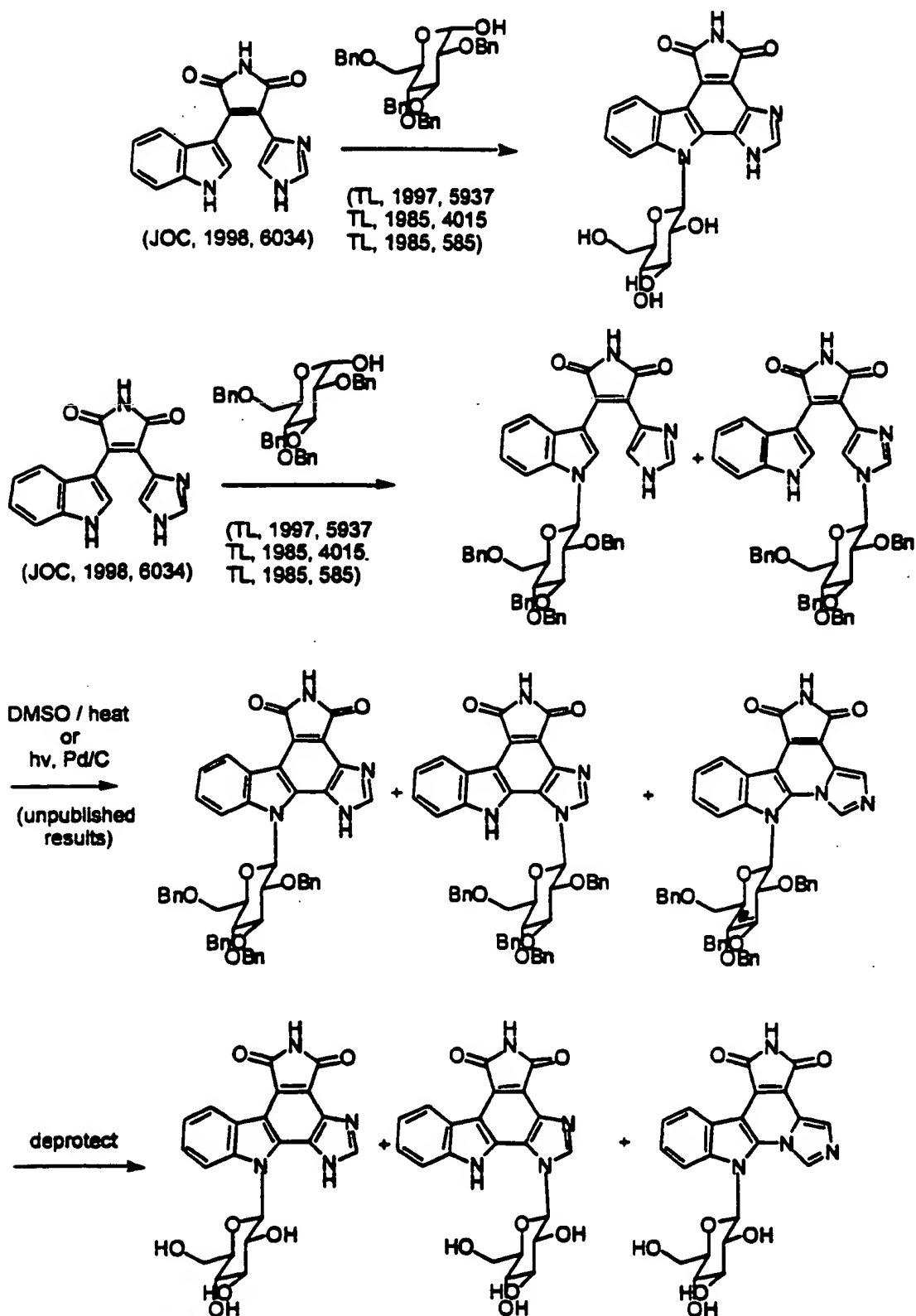
Example 9

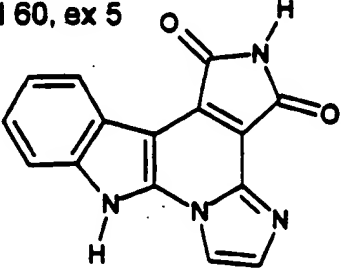
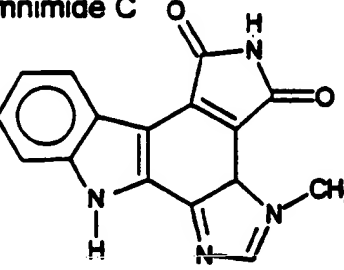
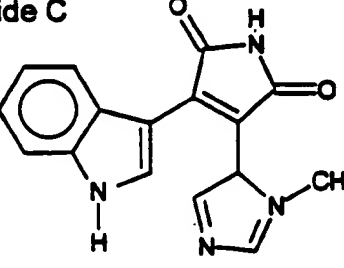
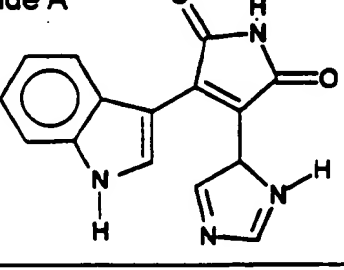
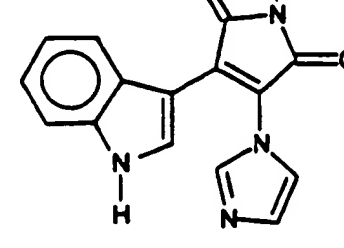
Synthesis of Compounds related to Staurosporine.



Example 10

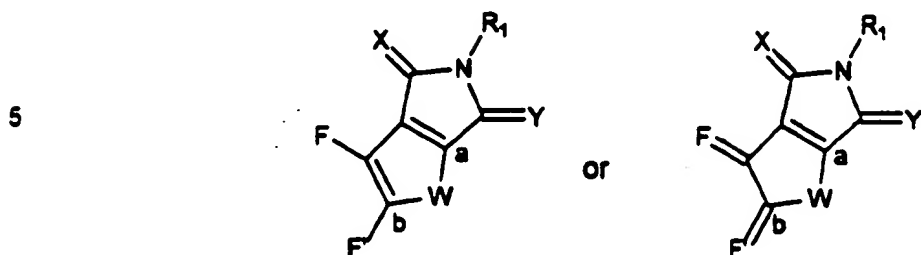
Synthesis of Compounds related to Rebeccamycin.



Compound 60, ex 5 RAB-007		6 μ M	17 μ M	5 μ M	>50 μ M
Cyclodidemnimide C Cmpd 70 RAB-005		inactive	>50 μ M	>50 μ M	>50 μ M
Didemnimide C RAB-006		inactive	5 μ M	1 μ M	17 μ M
Didemnimide A RAB-004		inactive	10 μ M	0.6 μ M	0.6 μ M
RAB-011		inactive	>50 μ M	>50 μ M	>50 μ M

WHAT IS CLAIMED IS:

1. A granulatinide compound having the structure:

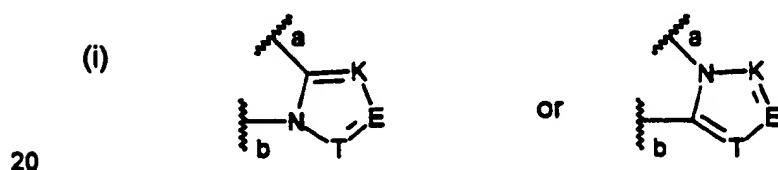


wherein:

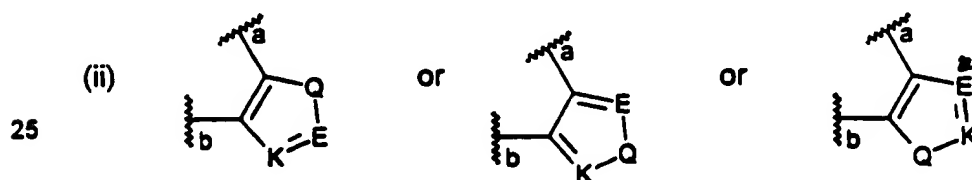
- 10 F and F' are independently R or Z as defined below, or in combination F and F' is Ar₁ as defined below;

Ar₁ is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings, optionally substituted with R or Z;

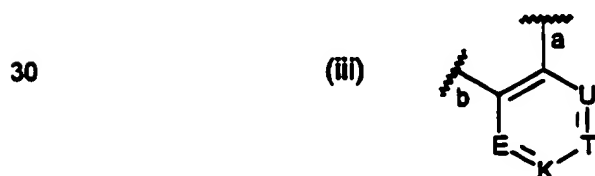
- 15 W is selected from the group consisting of formula (i); (ii) or (iii), wherein the structures are as follows:



in which K, E and T are independently selected from the group consisting of: N, CR, and CZ, and wherein R and Z are as defined below;



in which K and E are independently selected from the group consisting of: N, CR and CZ, and wherein R, Z and Q are as defined below; and



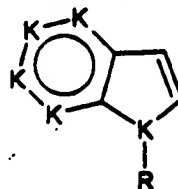
5. The granulatimide compound of Claim 3, wherein R_1 is H or CH_3 .

6. The granulatimide compound of Claim 1, wherein X and Y are oxygen.

5

7. The granulatimide compound of Claim 1, wherein F and F' are in combination, a bicyclic aromatic system comprising a five-membered and a six-membered ring, having the structure:

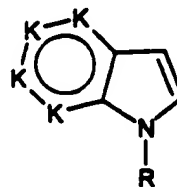
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wherein each K is independently is selected from the group consisting of N, CR and CZ; and wherein Z and R are as defined in Claim 1.

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8. The granulatimide compound of Claim 7, wherein F and F' in combination have the bicyclic structure:

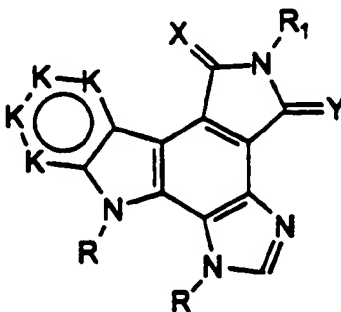


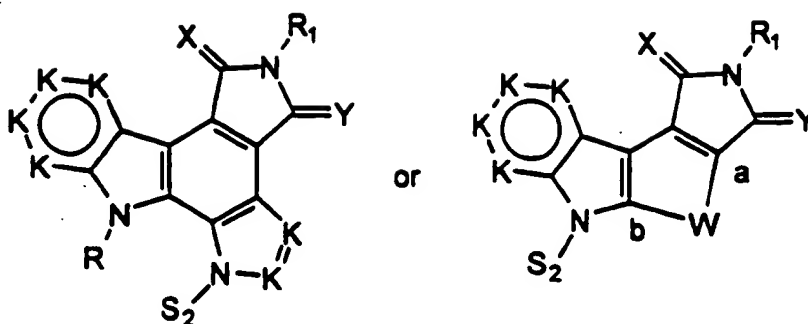
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9. The granulatimide compound of Claim 8, wherein W is a five-membered ring of formula (i) or formula (ii).

10. The granulatimide compound of Claim 9, having the structure:

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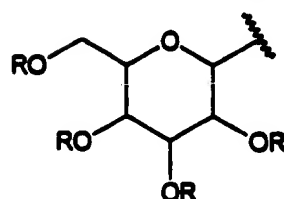




where K, X, Y and R₁ are as defined in Claim 1, R is H or an alkyl of from one to six carbon atoms; and S₂ has the formula (iv)

5

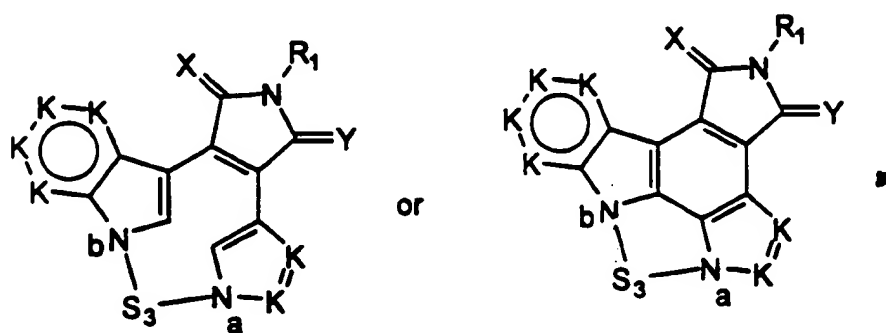
(iv)



or S₂ is a linear alkyl chain of from one to eight carbon atoms containing a terminal NR₂, where R₂ is an alkyl group of from 1 to 6 carbon atoms, branched, linear or cyclic.

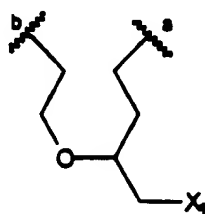
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13. The granulatimide compound of Claim 10, wherein the compound is further derivatized as follows:



where K, X, Y and R₁ are as defined in Claim 1; S₃ is

15



20. A method of inhibiting a protein kinase, the method comprising:
contacting said protein kinase with a dose of a granulatimide compound of Claim 1, in an amount effective to inhibit said protein kinase.
- 5 21. A method of treating a condition characterized by a defect in protein kinase mediated signaling, the method comprising:
administering a dose of a granulatimide compound of Claim 1, in an amount effective to inhibit said protein kinase.

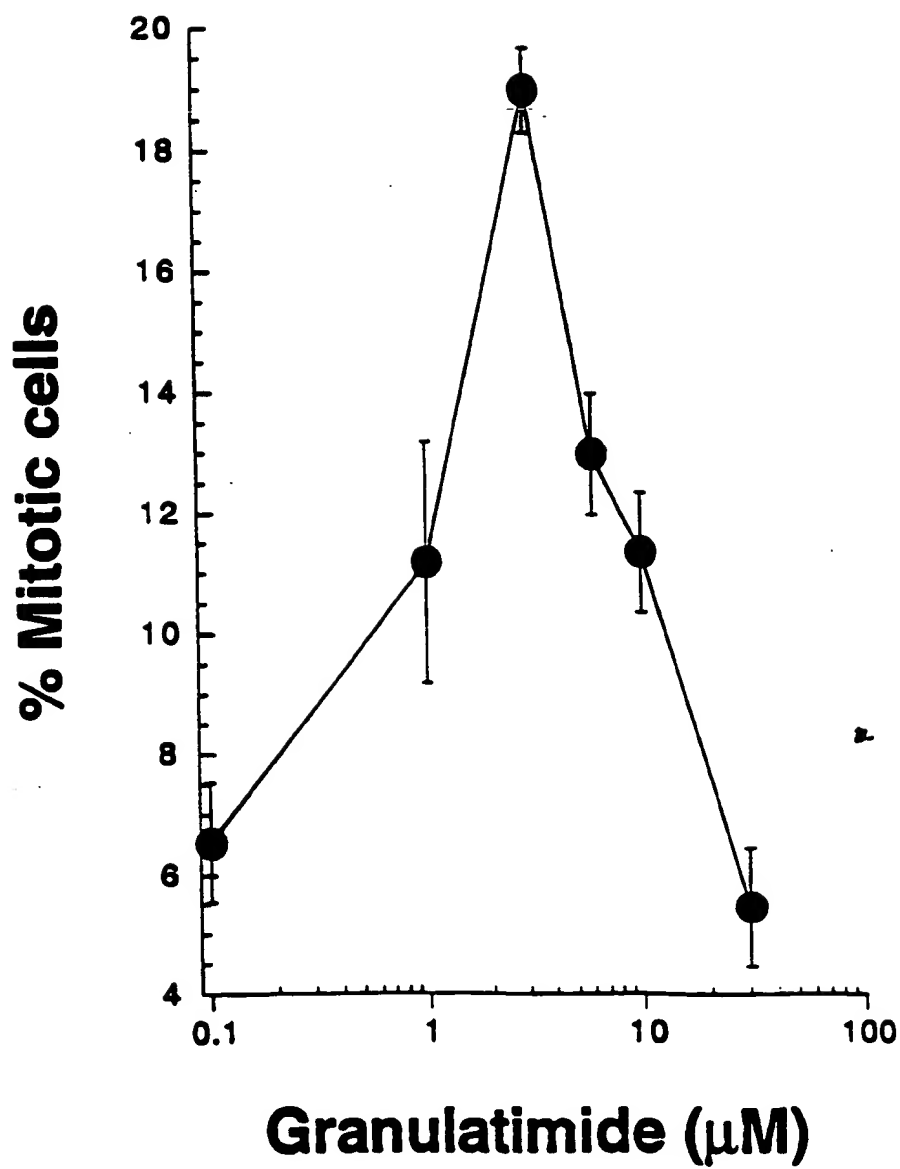


FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/00224

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 97 19080 A (LILLY CO ELI ;FAUL MARGARET M (US); KRUMRICH CHRISTINE A (US); WIN) 29 May 1997 see claim 1 ---	13
A	EP 0 735 038 A (LILLY CO ELI) 2 October 1996 see claim 1 ---	13
A	WO 96 11933 A (CEPHALON INC) 25 April 1996 see claim 1 ---	1
A	WO 95 32976 A (CIBA GEIGY AG ;REGENASS URS (CH); CARAVATTI GIORGIO (CH); WACKER O) 7 December 1995 see claim 1 ---	13
A	WO 95 32974 A (CIBA GEIGY AG ;REGENASS URS (CH); CARAVATTI GIORGIO (CH); WACKER O) 7 December 1995 see claim 1 ---	11
A	WO 95 32975 A (CIBA GEIGY AG ;REGENASS URS (CH); CARAVATTI GIORGIO (CH); FREDENHA) 7 December 1995 see claim 1 ---	13
A	EP 0 672 668 A (KYOWA HAKKO KOGYO KK) 20 September 1995 see claim 1 ---	11
A	EP 0 657 458 A (LILLY CO ELI) 14 June 1995 see claim 1 ---	13
A	WO 94 07895 A (SCHERING CORP ;VICE SUSAN F (US)) 14 April 1994 see claim 1 ---	13
A	WO 94 04541 A (UPJOHN CO ;ABRAHAM IRENE (US); ARISTOFF PAUL A (US); SKULNICK HARV) 3 March 1994 see claim 1 ---	11
A	WO 91 09034 A (SCHERING CORP) 27 June 1991 see claim 1 ---	1
A	EP 0 434 057 A (GOEDECKE AG) 26 June 1991 see claim 1 ---	1
A	DE 38 35 842 A (GOEDECKE AG) 26 April 1990 see claim 1 ---	1
A	EP 0 328 000 A (GOEDECKE AG) 16 August 1989 see claim 1 -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 99/00224

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0841337 A	13-05-1998	FR 2755691 A	15-05-1998
		AU 4440497 A	14-05-1998
		CA 2220995 A	08-05-1998
		CN 1182087 A	20-05-1998
		HU 9701922 A	28-12-1998
		JP 10139779 A	26-05-1998
		NO 974985 A	11-05-1998
		NZ 329134 A	25-03-1998
		PL 323033 A	11-05-1998
		US 5807882 A	15-09-1998
WO 9718809 A	29-05-1997	AT 172199 T	15-10-1998
		AU 1054897 A	11-06-1997
		CA 2237221 A	29-05-1997
		CN 1202825 A	23-12-1998
		CZ 9801502 A	16-12-1998
		DE 69600784 D	19-11-1998
		EP 0776895 A	04-06-1997
		ES 2122764 T	16-12-1998
		JP 11500149 T	06-01-1999
		NO 982182 A	13-05-1998
		NZ 323571 A	23-12-1998
		PL 326754 A	26-10-1998
		SI 776895 T	30-04-1999
WO 9719080 A	29-05-1997	AU 701659 B	04-02-1999
		AU 7738896 A	11-06-1997
		CA 2237401 A	29-05-1997
		CN 1207740 A	10-02-1999
		CZ 9801503 A	16-12-1998
		EP 0776899 A	04-06-1997
		NO 982105 A	08-05-1998
		NZ 323282 A	28-01-1999
		PL 326753 A	26-10-1998
EP 0735038 A	02-10-1996	US 5624949 A	29-04-1997
		AU 701988 B	21-02-1999
		AU 5324996 A	16-10-1996
		CA 2216535 A	03-10-1996
		CN 1185742 A	24-06-1998
		CZ 9703051 A	13-05-1998
		HU 9801250 A	28-09-1998
		NO 974453 A	19-11-1997
		NZ 305276 A	25-02-1999
		PL 322584 A	02-02-1998
		WO 9630048 A	03-10-1996
		US 5552396 A	03-09-1996
		US 5674862 A	07-10-1997
		US 5621098 A	15-04-1997
		US 5780461 A	14-07-1998
		US 5696108 A	09-12-1997
		US 5719175 A	17-02-1998
		US 5723456 A	03-03-1998
		US 5739322 A	14-04-1998
		US 5843935 A	01-12-1998
		US 5821365 A	13-10-1998
WO 9611933 A	25-04-1996	US 5475110 A	12-12-1995

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CA 99/00224

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0657458 A		US 5723456 A	03-03-1998
		US 5698578 A	16-12-1997
		US 5739322 A	14-04-1998
		US 5843935 A	01-12-1998
		US 5821365 A	13-10-1998
		ZA 9409611 A	03-06-1996
		BR 9502611 A	01-10-1996
WO 9407895 A	14-04-1994	AU 5100393 A	26-04-1994
		CN 1088211 A	22-06-1994
		US 5589472 A	31-12-1996
		ZA 9307042 A	05-01-1995
WO 9404541 A	03-03-1994	AU 4787693 A	15-03-1994
		CA 2140653 A	03-03-1994
		EP 0655066 A	31-05-1995
		JP 8500112 T	09-01-1996
WO 9109034 A	27-06-1991	AU 7035991 A	18-07-1991
		US 5618809 A	08-04-1997
EP 0434057 A	26-06-1991	DE 3942296 A	27-06-1991
		AT 140920 T	15-08-1996
		DE 59010435 D	05-09-1996
		DK 434057 T	25-11-1996
		ES 2090081 T	16-10-1996
		GR 3020866 T	30-11-1996
		JP 3294279 A	25-12-1991
		US 5489608 A	06-02-1996
DE 3835842 A	26-04-1990	AT 70839 T	15-01-1992
		EP 0370236 A	30-05-1990
		GR 3003452 T	17-02-1993
		JP 2174778 A	06-07-1990
		US 5438050 A	01-08-1995
EP 0328000 A	16-08-1989	DE 3803620 A	27-08-1989
		AT 117304 T	15-02-1995
		AU 2960789 A	10-08-1989
		CA 1337767 A	19-12-1995
		CN 1035667 A	20-09-1989
		DD 283394 A	10-10-1990
		DE 58908892 D	02-03-1995
		DK 51489 A	07-08-1989
		ES 2066798 T	16-03-1995
		GR 3015194 T	31-05-1995
		IE 65256 B	18-10-1995
		JP 2149520 A	08-06-1990
		JP 2866096 B	08-03-1999
		PT 89623 A,B	04-10-1989
		US 5438050 A	01-08-1995